# A GUM (LEVAN) BACTERIUM FROM A SACCHARINE EXUDATE OF *EUCALYPTUS STUARTIANA*.

(*Bacterium eucalypti*, n.sp.)

By R. Greig Smith, M.Sc., Macleay Bacteriologist to the Society.

(Plate xii.)

A sweet exudate from a species of Eucalyptus was upon examination found to contain a quantity of gum precipitable by alcohol, and, as several gum-forming bacteria had been under investigation in the Society's laboratory, the specimen was tested to see if the gum could possibly have a microbic origin. Plates of saccharose-gelatine* were infected in the usual manner, and upon these there developed the dome-shaped colonies so characteristic of many gum bacteria.

The exudate was a pale straw-coloured syrup, very similar in appearance and consistency to honey or golden syrup, and had fragments of bark, Eucalyptus capsules, etc., scattered throughout the mass. When dissolved in water and separated from woody débris, a portion contained :—

| | |
|---|---|
| Non-reducing but hydrolysable sugar† calculated to saccharose ... ... ... | 1·1 grm. |
| Reducing sugars ... ... ... ... | 2·5 ,, |
| Crude gum ... ... ... ... ... | 0·8 ,, |

* Saccharose 10, peptone 0·25, potassium chloride 0·5, sodium phosphate 0·2, gelatine 10, water to 100. Acidity to phenolphthalein 10 c.c. = 0·1 c.c. tenth normal acid.

† This is probably raffinose, the sugar of Eucalyptus manna. The reducing sugars probably consist of a mixture of levulose and melibiose.

I obtained the specimen from Mr. J. H. Maiden, Government Botanist. It had been taken from the bark of a *Eucalyptus Stuartiana*, F.v.M., by Mr. A. M. N. Rose at Dalgety, Southern Monaro. Mr. Maiden obtained for me two more samples from the same tree. The second specimen consisted of the exudate *in situ* adhering to the bark, and containing fragments of a ruby-coloured kino. The third specimen consisted of a mixture of the same exudate with Eucalyptus manna of various colours ranging from white to reddish-brown. In portions of the white manna I found small quantities of the same gum that was obtained from the first exudate, and after separating the gum spherical masses of prismatic crystals of raffinose were readily obtained.

In all three specimens the same bacterium was obtained in practically pure culture.

A quantity of the gum was prepared by growing the bacterium in saccharose-peptone fluid, and after a sufficient amount had been formed, as indicated by the medium being very opalescent, the gum was precipitated with alcohol, and purified by repeated solution in water and precipitation with alcohol. When free from reducing sugars the gum was tested with the following results. Fehling's solution was not reduced, and the gum readily hydrolysed with dilute acids producing a reducing sugar which yielded glucosazone. Basic lead acetate gave a strong opalescence, and the solution passed through filter paper unaltered. Ammoniacal lead acetate, barium hydrate, strontium hydrate and lime water in excess, each gave a white precipitate. Lead acetate, tannic acid, ferric chloride, copper sulphate, aluminium hydrate, iodine, sodium hydrate and ammoniacal silver nitrate gave no reaction. The melting point of the dry and powdered gum was 199° C. Mr. T. U. Walton, B.Sc., of the Colonial Sugar Refining Co., found the sugar to be laevorotatory, and to hydrolyse completely to levulose.

From these results it is evident that the gum is levan, which I first obtained on cultivating *Bac. levaniformans* in saccharose media.

The bacterium differs absolutely morphologically from *Bac. levaniformans*, the organism which occurs in cane-juice, and in raw and refined sugars; and it is interesting that the same gum should be formed by two widely differing species of bacteria. The specific characters of the bacterium, which I have named *Bacterium eucalypti*, are given at the end of this paper.

In testing the various points connected with the growth of the organism, it was found that growth occurred at 22°, 30°, and 37° C. The bacterium did not appear to have a preference for either of the higher temperatures; the growths appeared equally copious, although at 37° it was dry and stiff, while at 30° it was moist and flowing. A faintly acid medium (acidity = 0·075% tartaric acid) enables the organism to grow better, and to produce more gum than neutral or slightly alkaline media. Saccharose and raffinose (*e.g.*, Eucalyptus manna) are the only carbohydrates from which the bacterium appears to form gum. No levan was produced, and the growth was always scanty when dextrin, starch, levulose, dextrose, lactose or maltose was substituted for saccharose in the medium.

The composition of the fluid saccharose culture* as regards sugars and gum was tested in the manner described in a former paper† at the end of one, nine and nineteen days. The results are calculated upon 100 parts of saccharose which the medium contained in the litre, and due allowance has been made for the evaporation of the culture fluid during its incubation at 30°.

PRODUCTION OF LEVAN AND REDUCING SUGARS FROM SACCHAROSE.

| Temperature = 30° C. ... | At Start. | 1 day. | 9 days. | 19 days. |
|---|---|---|---|---|
| Saccharose ... ... ... | 100 | 95·2 | 11·4 | 3·8 |
| Reducing sugars ... ... | — | 1·5 | 54·1 | 63·2 |
| Levan ... ... ... | — | 2·8 | 33·4 | 31·8 |

* Saccharose 100, peptone 10, potassium chloride 5, sodium phosphate 2, tap water to 1000.

† These Proceedings, 1901, Pt. iv., 593.

These results show that the action of this bacterium upon saccharose is precisely similar to that of *Bac. levaniformans.* The relative amounts of levan and reducing sugars are about the same, and there is also a similar hydrolysis of the levan by the secreted acids in the old culture. On the 19th day the culture medium contained an acidity equal to 0·135% of lactic acid.

For the same reason that *levaniformans* was shown to secrete invertase, so is it with this bacterium; the amount of acids secreted in both cases is similar, and too small to account for the heavy inversion, which must, therefore, be ascribed to the action of an enzyme.

During the bacterial fermentation, carbon dioxide is evolved. This was made manifest by connecting the cultivation flask with a bottle containing baryta water and aspirating air which had passed over soda-lime, through the apparatus. A copious formation of barium carbonate occurred.

The acids secreted by the bacterium were tested in the manner already described for *Bac. levaniformans.** The chief acid formed is lactic; capric, formic and acetic acids were detected. The presence of butyric acid could not be definitely proved, which may be accounted for by the fact that a young (6 days') culture was used for the separation, and it is admitted in some cases of butyric fermentation that the butyric acid is formed from the calcium salt of lactic acid, which means that it is formed at a later stage of the fermentation.

It would appear that in the presence of calcium carbonate the reducing sugars are used for the formation of acid. A chalk culture which had been incubated for 12 days at 30° contained the following constituents per litre :—

| | |
|---|---|
| Saccharose ... ... ... ... .. | 12 grms. |
| Mixed reducing sugars ... ... ... | 18 ,, |
| Levan .. ... ... ... ... | 32 ,, |

The saccharose and levan are in the proportions and amount which were found under ordinary conditions of cultivation both

* These Proceedings, 1901, Pt. iv., p. 605 *et seq.*

with this bacterium and with *Bac. levaniformans*, but instead of the calculated 55 grms. of reducing sugars which should have been present there are only 18 grms.; the difference (37 grms.) has disappeared, *i.e.*, it has been converted into acids.

Although levan can be formed from saccharose, it must not be forgotten that the gum found naturally in the exudate had in all probability been formed from raffinose, the sugar of Eucalyptus manna. This is indicated by the presence of manna in one of the samples. That levan could be produced by the organism from raffinose is to be expected from the fact found in the study of *Bac. levaniformans*, viz., that the gum was formed chiefly from nascent levulose, and from the fact that raffinose under the influence of invertase splits up into levulose and melibiose.

### Bacterium eucalypti, n.sp.

*Shape, etc.*—An actively motile, short *coli*-like bacterium, measuring generally in the stained and imbedded condition $0{\cdot}5 : 1\ \mu$. It stains well with violet and fuchsin, but feebly with blue: it is decolourised by the Gram method. The flagella are long, and vary in number from one to nine, and are studded over the surface of the cell. No spores are formed.

*Relation to temperature, etc.*—The bacterium is aërobic, and appears to grow equally well at 28° and at 37°.

*Nutrient gelatine plate.*—Small punctiform colonies are visible in seven days, and by the eleventh day they have become rounded, translucent white, and 3 mm. in diameter. When magnified they appear round or rounded, and finely granular, sometimes with central granules. The edge is smooth and slightly waved. The deep colonies are irregular and finely granular.

*Glucose-gelatine plate.*—The colonies are glistening, translucent white and rounded. When magnified they appear rounded and erose with *coli*-like striations. The deep colonies are rounded to elliptical, and have a striated margin.

*Wort*-gelatine.*—The colonies are raised and like drops of whey. When magnified they appear round and uniformly granular. Sometimes the margin is striated as if from the growth flowing down the dome-shaped colony. The deep and subsurface colonies are small and coarsely granular.

*Saccharose-gelatine plate.*—The colonies are transparent and hemispherical, like exuded drops of glycerine.

*Nutrient agar plate.*—The colonies are round, slightly raised, translucent white and moist glistening. When magnified they appear round with a smooth edge. There are granules around the centre, but otherwise the structure appears homogeneous. The deep colonies are rounded to elliptical, and contain large granules.

*Saccharose-peptone-agar plate.*—The colonies are hemispherical and whitish, like drops of starch paste. When magnified they appear rounded, and have a blistered surface; the margin is apparently smooth.

*Nutrient gelatine stab.*—The stab is filiform and white; the nail-head is round, flat, white and glistening. The medium is slowly liquefied; in 14 days at 22° the liquefied area is slightly funicular, and at the top of the stab the medium has been consumed, leaving an air-bubble.

*Glucose-gelatine stab.*—As with nutrient gelatine.

*Saccharose-gelatine stab.*—A filiform stab with an hemispherical drop of transparent fluid as a nail-head. The fluid increases and flows over the surface of the gelatine. As this occurs, the stab develops lenticular and spherical pockets of almost transparent gum. A liquefaction of the medium below the surface was noted after a month.

*Glucose-gelatine stroke.*—The growth is rough and narrow, with an expanded lower portion like the nail-head on nutrient gelatine. The stroke becomes rough and ribbed, dry glistening and whitish.

*Nutrient agar stroke.*—A thin translucent white, almost transparent, layer is formed. It is always scanty, and the condensed water has a white sediment and no film.

---

* The wort contained saccharose.

*Saccharose-peptone agar.*—The stroke becomes broad, raised or hemispherical in section, sometimes undulating, and slowly gravitates. The culture is of the appearance and consistency of thin starch paste. The luxuriance of the growth is in striking contrast to the growth on nutrient agar.

*Potato.*—The growth is dry and glistening, whitish or slightly yellow and constricted. The colour deepens to cream or pale buff, and the growth becomes raised. It is always meagre, and does not spread over the surface of the medium.

*Bouillon.*—The medium becomes turbid, and a fine white, loose sediment is deposited, while a slight film forms on the surface. A faint indol reaction is obtained In nitrate-bouillon the nitrate is not reduced.

*Milk.*—The medium is unaltered.

*Saccharose-peptone fluid.*—The medium becomes milk-white, and at a later stage becomes brownish-yellow. The gum can be readily precipitated by alcohol, and Fehling's solution is strongly reduced.

The nearest allied bacterium capable of forming slime from saccharose appears to be *Bact. gelatinosum betæ*, Glaser, which forms dextran and alcohol, but no lactic acid. As the gum, moreover, has only been obtained previously from *Bac. levaniformans* the bacterium is evidently a new species, and therefore I have named it, on account of its origin, *Bacterium eucalypti.*

---

## EXPLANATION OF PLATE XII.

Film of culture on saccharose-peptone-agar, stained with carbol-violet. × 1000.

Flagella stained by the night-blue method; bacteria grown on ordinary nutrient agar. × 1000.

Dome-shaped colonies on saccharose-gelatine plate. × $\frac{1}{2}$.